

Phylogenetic Characterization and Description of Novel Heat-tolerant *Bacillus* species Isolated from Spacecraft Assembly Facility

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Forward contamination of samples with cells or biomarkers from Earth would seriously compromise interpretation of results of a Mars sample return mission. In ongoing investigations to map and archive the microbial footprints in various components of spacecraft and its accessories, we have examined the microbial populations of the Spacecraft Assembly Facility (SAF). The dimension of the SAF High Bay-1 is 80 feet wide x 120 feet long x 44 feet 4 inches high. Relative humidity is controlled at 40±5% with a cap at 45% and the average temperature is maintained at 20±5°C. We have exposed witness plates that are made up of spacecraft materials and or painted with spacecraft-quality paints for ~7 to 9 months. In the initial studies reported here, we have examined the total cultivable aerobic heterotrophs, and heat-tolerant (80°C for 15-min.) spore-formers. The particles that were collected by natural fall-out were in the size range of 11 to 150 µm. Fibers of various sizes (100 through 1,500 µm) were also noticed. The results showed that the witness plates coated with spacecraft quality paints attracted more dust particles than the non-coated stainless steel witness plates. Among four paints tested, witness plates coated with NS43G (an off-white conductive paint [silicate binder]) accumulated the highest number of particles, hence attracted more cultivable microbes and spore-formers. The microbiological examination revealed that the SAF High Bay-1 harbors mainly Gram-positive microbes and mostly spore-forming *Bacillus* species. Most of the isolated microbes were heat resistant to 80°C and grew well at 60°C. Based on the morphology, and physiology, 28 isolates were chosen for further study. The phylogenetic relationships among these heat-tolerant microbes were examined using a battery of morphological, physiological, molecular and chemotaxonomic characterizations. Using phenotypic characterization, only 9 strains were identified. Sequence analysis of nearly complete sequences of 16S ribosomal RNA revealed that most of the microbes in SAF are *Bacillus licheniformis*. By 16S rRNA analysis, the isolates fell into seven clades: *Bacillus licheniformis*, *B. pumilus*, *B. cereus*, *B. circulans*, *Staphylococcus epidermis*, *Planococcus* sp. and *Micrococcus lylae*. In addition to the *Bacillus* species (22 isolates), the isolation of human-associated microbes such as *S. epidermis* indicates secondary contamination by human activity in SAF.

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